

## **REMARKS/ARGUMENTS**

The foregoing amendments in the specification and claims are of a formal nature, and do not add new matter.

Prior to the present amendment, Claims 28-47 were pending in this application and were rejected on various grounds. With this amendment, Claims 36-37 and 41-43 have been canceled without prejudice, Claims 28-35, 38-39 and 44 have been amended to clarify what Applicants have always regarded as their invention, and new Claims 48-62 have been added.

Claims 28-35, 38-40 and 44-62 are pending after entry of the instant amendment. Applicants expressly reserve the right to pursue any canceled matter in subsequent continuation, divisional or continuation-in-part applications.

The amendments to the specification and claims are fully supported by the specification and claims as originally filed and do not constitute new matter. In addition, new Claims 48-62 are fully supported by the specification as originally filed. Amendments to Claims 28-32 can be found in Example 153 at least on page 514, line 5 of the specification. Support new Claims 48-52 can be found at least in Example 150 at least on page 512, line 12 of the specification. Support for new Claims 53-57 can be found at least in Example 149, starting on page 511, line 34 of the specification. Support for new Claims 58-62 can be found at least in Example 146, starting on page 509, line 37 of the specification.

### **1. Formal Matters**

Applicants thank the Examiner for entering the Preliminary Amendments filed on December 14, 2001 and August 29, 2002 into the record. Applicants also thank the Examiner for entering the Information Disclosure Statement filed on September 20, 2002 into the record.

### **2. Priority**

The Examiner alleges that "[d]ue to the excessive number of applications from which the present application claims benefit, priority cannot be determined." Thus, the Examiner asserts that the effective filing date for the application is September 4, 2001, which is the filing date of

the parent application 09/946,374.

The Examiner's attention is respectfully directed to the Preliminary Amendment filed on August 29, 2002, which states that the present application is "a continuation of, and claims priority under 35 U.S.C. §120 to, U.S. Application 09/946,374 filed 9/4/2001, which is a continuation of, and claims priority under 35 U.S.C. §120 to, PCT Application PCT/US00/04342 filed 2/18/2000, which is a continuation-in-part of, and claims priority under 35 U.S.C. §120 to, U.S. Patent Application 09/403,297 filed 10/18/1999, now abandoned, which is the National Stage filed under 35 U.S.C. §371 of PCT Application PCT/US99/20111 filed 9/1/1999, which claims priority under 35 U.S.C. §119 to US Provisional Application Serial No. 60/101,741 filed 9/24/1998."

As discussed below, Applicants further rely on the chondrocyte proliferation assay (Example 153, Assay 111), chondrocyte re-differentiation assay (Example 150, Assay 110), the glucose/FFA uptake assay (Example 149, Assay 94) and induction of pancreatic  $\beta$ -cell precursor differentiation assay (Example 146, Assay 89) for patentable utility which were first disclosed in PCT/US00/04342 filed on February 18, 2000, priority to which has been claimed in this application. Accordingly, the present application is entitled to at least the February 18, 2000 priority. In support, Applicants enclose herewith page 525, describing the chondrocyte proliferation assay (Example 153), page 523, describing the chondrocyte re-differentiation assay (Example 150) and the glucose/FFA uptake assay (Example 149) and pages 521-522 describing the induction of pancreatic  $\beta$ -cell precursor differentiation assay (Example 146) of the PCT Publication WO 00/78961, corresponding to PCT Application PCT/US00/04342.

### **3. Information Disclosure Statement**

In response to the Examiner's assertion that references 1 and 2 in the Information Disclosure Statement filed on September 20, 2002 are not in proper format, Applicants file herewith, an Information Disclosure Statement listing each reference of the "Blast Search" separately and including authors/inventors, relevant accession numbers and publication dates. Applicants respectfully request that the listed information be considered by the Examiner and be

made of record in the above-identified application.

**4. Specification**

As requested by the Examiner, the specification has been amended to remove embedded hyperlink and/or other form of browser-executable code.

The title of the application has been amended to recite a new, descriptive title indicative of the invention to which the claims are directed.

**5. Claim Objections**

Claims 28-47 were objected to for reciting a Figure number and a SEQ ID NO. Applicants submit that the cancellation of Claims 36-37 and 41-43 renders the objection to these claims moot. Further, Applicants submit that Claims 28-35 and 38-39 have been amended to only recite SEQ ID NO. Accordingly, Applicants respectfully request that the Examiner withdraw the objection to Claims 28-35, 38-40 and 44-47.

**6. Claim Rejections Under 35 U.S.C. §112, First Paragraph (Enablement)**

A. Claims 28-47 stand rejected under 35 U.S.C. §112, first paragraph, allegedly for "containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention." The Examiner specifically notes that "the deposit of the biological material is considered necessary for the enablement of the current invention."

Applicants submit that the cancellation of Claims 36 and 37 renders the rejection of these claims moot.

Applicants disagree with the Examiner's assertion that the deposit was necessary for enablement of the current invention. The current invention is fully enabled by the disclosure of the present application, including the sequence of PRO1474 and its coding sequence. Further, as discussed above, the foregoing amendment to the specification corrects the address of ATCC, and further elaborates on the conditions of the deposit, which was made for patent purposes, under the terms of the Budapest treaty.

Nevertheless, Applicants enclose herewith a copy of the deposit receipt indicating that DNA73739-1645 deposit, ATCC Deposit No. 203270, was made by Applicants on September 22, 1998.

In addition, as stated above, Applicants respectfully submit that the specification clearly discloses that the deposit was made under the Budapest Treaty and provides the accession number for the deposit, the date of the deposit, the description of the deposited material, and the name and address of the depository starting on page 517, line 1 of the specification.

Applicants further submit that the specification has been amended to recite that the deposit will be maintained "for 30 years from the date of deposit and for at least five (5) years after the most recent request for the furnishing of a sample of the deposit received by the depository" and to recite that "all restrictions imposed by the depositor on the availability to the public of the deposited material will be irrevocably removed upon the granting of the pertinent U.S. patent."

Accordingly, Applicants believe that the present rejection should be withdrawn.

**B.** The Examiner further alleges that even if Claims 28-47 possessed utility under 35 U.S.C. §101, which Applicants assert they do, "Claims 28-47 would still be rejected under 35 U.S.C. §112, first paragraph, because the specification, while then being enabling for SEQ ID NO:333 and 334, does not reasonably provide enablement for polynucleotides or polypeptides having at least 80%, 85%, 90%, 95% or 99% sequence identity to SEQ ID NO:333 or 334, to the protein encoded by ATCC No. 203270, for the extracellular domain thereof, or for vectors and host cells containing these polynucleotides." In addition, the Examiner alleges that "[t]he claims are too broad ... because the claims have no functional limitation."

Applicants respectfully disagree and traverse the rejection.

Without acquiescing to the Examiner's position, and solely in the interest of expediting prosecution in this case, Claims 28-32 (and, as a consequence, those claims dependent from the same) have been amended to recite a functional limitation that the encoded polypeptide "induces chondrocyte proliferation." Applicants submit that the specification provides ample enablement

for such polypeptides based on the *in vitro* data provided in the chondrocyte proliferation example (Example 153). Coupled with the general knowledge in the art at the time of the invention, Applicants submit that the present application provides sufficient guidance to one skilled in the art to use the invention without undue experimentation. As the M.P.E.P. states, "The fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *In re Certain Limited-charge cell Culture Microcarriers*, 221 USPQ 1165, 1174 (Int'l Trade Comm'n 1983), *aff. sub nom.*, *Massachusetts Institute of Technology v A.B. Fortia*, 774 F.2d 1104, 227 USPQ 428 (Fed. Cir. 1985) M.P.E.P. 2164.01. The Examiner is therefore, respectfully requested to reconsider and withdraw the rejection of these claims under 35 U.S.C. §112, first paragraph.

**7. Claim Rejections Under 35 U.S.C. §112, First Paragraph (Written Description)**

Claims 28-47 are rejected under 35 U.S.C. §112, first paragraph, for allegedly containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. In particular, the Examiner notes that "[t]he claims are drawn to polynucleotides having at least 80%, 85%, 90%, 95% or 99% sequence identity with SEQ ID NO:333 as well as vectors and host cells[, without requiring] that the polynucleotides or encoded polypeptides of the present invention possess any particular biological activity . . . ."

Applicants submit that the cancellation of Claims 36-37 and 41-43 renders the rejection of these claims moot.

Without acquiescing to the propriety of this rejection, solely in the interest of expediting prosecution in this case, Applicants respectfully submit that amended Claims 28-32 (and, as a consequence, those claims dependent from the same) now recite a functional limitation that "the encoded polypeptide induces chondrocyte proliferation." Accordingly, it is no longer true that the claims are drawn to a genus of polypeptides defined by sequence identity alone. Coupled with the general knowledge available in the art at the time of the invention, the specification provides ample written support for such polypeptides in Example 153 (page 514 of the

specification) where assay for the ability of polypeptides to induce chondrocyte proliferation is described. Thus, based on the high percentage of sequence identity and the described method to assay for induction of chondrocyte proliferation, one skilled in the art would have known at the time of the invention, that the Applicants had possession of the claimed polypeptides and polynucleotides.

The Examiner is therefore respectfully requested to reconsider and withdraw the rejection of these claims for allegedly lacking written support.

**9. Claim Rejections Under 35 U.S.C. §112, Second Paragraph**

A. The Examiner asserts Claims 119-138 are rejected under 35 U.S.C. §112, second paragraph. However, Applicants respectfully submit that there are no Claims 119-138 in the instant application. Therefore, Applicants assume the Examiner was referring to Claims 28-47.

Claims 28-47 are rejected under 35 U.S.C. §112, second paragraph, for allegedly “being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.” The Examiner notes that “it is not clear whether or not [PRO1474] is a soluble protein.” Accordingly, the Examiner states that the limitation that the claimed protein comprises an “extracellular domain” and the recitation of “the extracellular domain ... lacking its associated signal sequence” is indefinite.

Without acquiescing to the propriety of this rejection, solely in the interest of expediting prosecution in this case, Applicants submit that the cancellation of Claims 36-37 and 41-43 renders the rejection of these claims moot. Further, terms “extracellular domain” or the “extracellular domain ... lacking its associated signal sequence” are no longer present in Claims 28-33 (and, as a consequence, those claims dependent from the same).

Accordingly, Applicants request that the rejection of Claims 28-35, 38-40 and 44-47 under 35 U.S.C. §112, second paragraph, be withdrawn.

B. Examiner alleges that Claims 41-43 are vague and indefinite since the claim recited “hybridizes” without the recitation of any conditions, or recites “stringent conditions” wherein these conditions are not know.

Without acquiescing to the propriety of this rejection, solely in the interest of expediting prosecution in this case, Applicants submit that the cancellation of Claims 41-43 renders the rejection of these claims moot. Hence, present rejection should be withdrawn.

**9. Claim Rejections Under 35 U.S.C. §102**

Claims 28-47 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Bandman *et al.*, U.S. Patent No. 5,581,987, issue date of December 22, 1998. The Examiner notes that "Bandman [*et al.*] teach a polynucleotide which is 55.8% identical to SEQ ID NO:333."

Applicants respectfully submit that the cancellation of Claims 36-37 and 41-43 renders the rejection of these claims moot.

Furthermore, as amended, Claims 28-33 (and, as a consequence, those claims dependent from the same) no longer claim "extracellular domain" or the "extracellular domain ... lacking its associated signal sequence." Accordingly, Applicants respectfully submit Claims 28-35, 38-40 and 44-47 are not anticipated by Bandman *et al.* Hence, the Examiner is respectfully requested to reconsider and withdraw the rejection of these claims under 35 U.S.C. §102.

**10. Conclusion**

In conclusion, the present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited. Should there be any further issues outstanding, the Examiner is invited to contact the undersigned attorney at the telephone number shown below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 08-1641 (referencing Attorney's Docket No. 39780-2830 P1C62).

Respectfully submitted,

Date: November 22, 2004

By:   
Anna L. Barry (Reg. No. 51,436)

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SV 2053504 v1  
11/20/04 4:43 PM (39780.2830)

The following polypeptide tested positive in this assay: PRO1265, PRO1244 and PRO1382.

**EXAMPLE 146: Induction of Pancreatic  $\beta$ -Cell Precursor Differentiation (Assay 89)**

This assay shows that certain polypeptides of the invention act to induce differentiation of pancreatic  $\beta$ -cell precursor cells into mature pancreatic  $\beta$ -cells and, therefore, are useful for treating various insulin deficient states in mammals, including diabetes mellitus. The assay is performed as follows. The assay uses  
 5 a primary culture of mouse fetal pancreatic cells and the primary readout is an alteration in the expression of markers that represent either  $\beta$ -cell precursors or mature  $\beta$ -cells. Marker expression is measured by real time quantitative PCR (RTQ-PCR); wherein the marker being evaluated is insulin.

The pancreata are dissected from E14 embryos (CD1 mice). The pancreata are then digested with  
 10 collagenase/dispase in F12/DMEM at 37°C for 40 to 60 minutes (collagenase/dispase, 1.37 mg/ml, Boehringer Mannheim, #1097113). The digestion is then neutralized with an equal volume of 5% BSA and the cells are washed once with RPMI1640. At day 1, the cells are seeded into 12-well tissue culture plates (pre-coated with laminin, 20 $\mu$ g/ml in PBS, Boehringer Mannheim, #124317). Cells from pancreata from 1-2 embryos are distributed per well. The culture medium for this primary culture is 14F/1640. At day 2, the media is removed  
 15 and the attached cells washed with RPMI/1640. Two mls of minimal media are added in addition to the protein to be tested. At day 4, the media is removed and RNA prepared from the cells and marker expression analyzed by real time quantitative RT-PCR. A protein is considered to be active in the assay if it increases the expression of the relevant  $\beta$ -cell marker as compared to untreated controls.

14F/1640 is RPMI1640 (Gibco) plus the following:

- 20 group A 1:1000
- group B 1:1000
- recombinant human insulin 10  $\mu$ g/ml
- Aprotinin (50 $\mu$ g/ml) 1:2000 (Boehringer manheim #981532)
- Bovine pituitary extract (BPE) 60 $\mu$ g/ml
- 25 Gentamycin 100 ng/ml

Group A : (in 10ml PBS)

- Transferrin, 100mg (Sigma T2252)
- Epidermal Growth Factor, 100 $\mu$ g (BRL 100004)
- Triiodothyronine, 10 $\mu$ l of 5x10<sup>-6</sup> M (Sigma T5516)
- 30 Ethanolamine, 100 $\mu$ l of 10<sup>-1</sup> M (Sigma E0135)
- Phosphoethalamine, 100 $\mu$ l of 10<sup>-1</sup> M (Sigma P0503)
- Selenium, 4 $\mu$ l of 10<sup>-1</sup> M (Aesar #12574)

Group C : (in 10ml 100% ethanol)

- Hydrocortisone, 2 $\mu$ l of 5X10<sup>-3</sup> M (Sigma #H0135)
- 35 Progesterone, 100 $\mu$ l of 1X10<sup>-3</sup> M (Sigma #P6149)
- Forskolin, 500 $\mu$ l of 20mM (Calbiochem #344270)

Minimal media:

RPMI 1640 plus transferrin (10 µg/ml), insulin (1 µg/ml), gentamycin (100 ng/ml), aprotinin (50 µg/ml) and BPE (15 µg/ml).

Defined media:

RPMI 1640 plus transferrin (10 µg/ml), insulin (1 µg/ml), gentamycin (100 ng/ml) and aprotinin (50 µg/ml).

5 The following polypeptides were positive in this assay: PRO1275 and PRO1474.

EXAMPLE 147: Fetal Hemoglobin Induction in an Erythroblastic Cell Line (Assay 107)

10 This assay is useful for screening PRO polypeptides for the ability to induce the switch from adult hemoglobin to fetal hemoglobin in an erythroblastic cell line. Molecules testing positive in this assay are expected to be useful for therapeutically treating various mammalian hemoglobin-associated disorders such as the various thalassemias. The assay is performed as follows. Erythroblastic cells are plated in standard growth medium at 1000 cells/well in a 96 well format. PRO polypeptides are added to the growth medium at a concentration of 0.2% or 2% and the cells are incubated for 5 days at 37°C. As a positive control, cells are treated with 100µM hemin and as a negative control, the cells are untreated. After 5 days, cell lysates are prepared and analyzed for the expression of gamma globin (a fetal marker). A positive in the assay is a gamma globin level at least 2-fold above the negative control.

The following polypeptides tested positive in this assay: PRO1478, PRO1265, PRO1412, PRO1279, PRO1304, PRO1306, PRO1418, PRO1410 and PRO1575.

20 EXAMPLE 148: Detection of Polypeptides That Affect Glucose and/or FFA Uptake in Skeletal Muscle (Assay 106)

This assay is designed to determine whether PRO polypeptides show the ability to affect glucose or FFA uptake by skeletal muscle cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of disorders where either the stimulation or inhibition of glucose uptake by skeletal muscle would be beneficial including, for example, diabetes or hyper- or hypo-insulinemia.

25 In a 96 well format, PRO polypeptides to be assayed are added to primary rat differentiated skeletal muscle, and allowed to incubate overnight. Then fresh media with the PRO polypeptide and +/- insulin are added to the wells. The sample media is then monitored to determine glucose and FFA uptake by the skeletal muscle cells. The insulin will stimulate glucose and FFA uptake by the skeletal muscle, and insulin in media without the PRO polypeptide is used as a positive control, and a limit for scoring. As the PRO polypeptide being tested may either stimulate or inhibit glucose and FFA uptake, results are scored as positive in the assay if greater than 1.5 times or less than 0.5 times the insulin control.

30 The following PRO polypeptides tested positive as either stimulators or inhibitors of glucose and/or FFA uptake in this assay: PRO1130, PRO1275, PRO1418, PRO1555 and PRO1787.

35

EXAMPLE 149: Detection of PRO Polypeptides That Affect Glucose or FFA Uptake by Primary Rat Adipocytes (Assay 94)

This assay is designed to determine whether PRO polypeptides show the ability to affect glucose or FFA uptake by adipocyte cells. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of disorders where either the stimulation or inhibition of glucose uptake by adipocytes would be beneficial including, for example, obesity, diabetes or hyper- or hypo-insulinemia.

In a 96 well format, PRO polypeptides to be assayed are added to primary rat adipocytes, and allowed to incubate overnight. Samples are taken at 4 and 16 hours and assayed for glycerol, glucose and FFA uptake. After the 16 hour incubation, insulin is added to the media and allowed to incubate for 4 hours. At this time, a sample is taken and glycerol, glucose and FFA uptake is measured. Media containing insulin without the PRO polypeptide is used as a positive reference control. As the PRO polypeptide being tested may either stimulate or inhibit glucose and FFA uptake, results are scored as positive in the assay if greater than 1.5 times or less than 0.5 times the insulin control.

The following PRO polypeptides tested positive as stimulators of glucose and/or FFA uptake in this assay: PRO1265, PRO1283, PRO1279, PRO1303, PRO1306, PRO1325, PRO1565 and PRO1567.

The following PRO polypeptides tested positive as inhibitors of glucose and/or FFA uptake in this assay: PRO1194, PRO1190, PRO1326, PRO1343, PRO1480, PRO1474, PRO1575 and PRO1760.

EXAMPLE 150: Chondrocyte Re-differentiation Assay (Assay 110)

This assay shows that certain polypeptides of the invention act to induce redifferentiation of chondrocytes, therefore, are expected to be useful for the treatment of various bone and/or cartilage disorders such as, for example, sports injuries and arthritis. The assay is performed as follows. Porcine chondrocytes are isolated by overnight collagenase digestion of articular cartilage of metacarpophalangeal joints of 4-6 month old female pigs. The isolated cells are then seeded at 25,000 cells/cm<sup>2</sup> in Ham F-12 containing 10% FBS and 4 µg/ml gentamycin. The culture media is changed every third day and the cells are then seeded in 96 well plates at 5,000 cells/well in 100µl of the same media without serum and 100 µl of the test PRO polypeptide, 5 nM staurosporin (positive control) or medium alone (negative control) is added to give a final volume of 200 µl/well. After 5 days of incubation at 37°C, a picture of each well is taken and the differentiation state of the chondrocytes is determined. A positive result in the assay occurs when the redifferentiation of the chondrocytes is determined to be more similar to the positive control than the negative control.

The following polypeptide tested positive in this assay: PRO1265, PRO1250, PRO1430, PRO1356, PRO1275, PRO1274, PRO1286, PRO1273, PRO1283, PRO1279, PRO1306, PRO1325, PRO1343, PRO1418, PRO1565, PRO1474, PRO1787, PRO1556 and PRO1801.

EXAMPLE 151: Induction of Pancreatic β-Cell Precursor Proliferation (Assay 117)

This assay shows that certain polypeptides of the invention act to induce an increase in the number of pancreatic β-cell precursor cells and, therefore, are useful for treating various insulin deficient states in mammals, including diabetes mellitus. The assay is performed as follows. The assay uses a primary culture of mouse fetal pancreatic cells and the primary readout is an alteration in the expression of markers that represent

**EXAMPLE 152: Proliferation of Rat Utricular Supporting Cells (Assay 54)**

This assay shows that certain polypeptides of the invention act as potent mitogens for inner ear supporting cells which are auditory hair cell progenitors and, therefore, are useful for inducing the regeneration of auditory hair cells and treating hearing loss in mammals. The assay is performed as follows. Rat UEC-4 utricular epithelial cells are aliquoted into 96 well plates with a density of 3000 cells/well in 200  $\mu$ l of serum-containing medium at 33°C. The cells are cultured overnight and are then switched to serum-free medium at 37°C. Various dilutions of PRO polypeptides (or nothing for a control) are then added to the cultures and the cells are incubated for 24 hours. After the 24 hour incubation, <sup>3</sup>H-thymidine (1  $\mu$ Ci/well) is added and the cells are then cultured for an additional 24 hours. The cultures are then washed to remove unincorporated radiolabel, the cells harvested and Cpm per well determined. Cpm of at least 30% or greater in the PRO polypeptide treated cultures as compared to the control cultures is considered a positive in the assay.

The following polypeptides tested positive in this assay: PRO1340.

**EXAMPLE 153: Chondrocyte Proliferation Assay (Assay 111)**

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to induce the proliferation and/or redifferentiation of chondrocytes in culture. PRO polypeptides testing positive in this assay would be expected to be useful for the therapeutic treatment of various bone and/or cartilage disorders such as, for example, sports injuries and arthritis.

Porcine chondrocytes are isolated by overnight collagenase digestion of articular cartilage of the metacarpophalangeal joint of 4-6 month old female pigs. The isolated cells are then seeded at 25,000 cells/cm<sup>2</sup> in Ham F-12 containing 10% FBS and 4  $\mu$ g/ml gentamycin. The culture media is changed every third day and the cells are reseeded to 25,000 cells/cm<sup>2</sup> every five days. On day 12, the cells are seeded in 96 well plates at 5,000 cells/well in 100  $\mu$ l of the same media without serum and 100  $\mu$ l of either serum-free medium (negative control), staurosporin (final concentration of 5 nM; positive control) or the test PRO polypeptide are added to give a final volume of 200  $\mu$ l/well. After 5 days at 37°C, 20  $\mu$ l of Alamar blue is added to each well and the plates are incubated for an additional 3 hours at 37°C. The fluorescence is then measured in each well (Ex:530 nm; Em: 590 nm). The fluorescence of a plate containing 200  $\mu$ l of the serum-free medium is measured to obtain the background. A positive result in the assay is obtained when the fluorescence of the PRO polypeptide treated sample is more like that of the positive control than the negative control.

The following PRO polypeptides tested positive in this assay: PRO1265, PRO1412, PRO1347, PRO1279, PRO1410 and PRO1474.

**EXAMPLE 154: Inhibition of Heart Neonatal Hypertrophy Induced by LIF+ET-1 (Assay 74)**

This assay is designed to determine whether PRO polypeptides of the present invention show the ability to inhibit neonatal heart hypertrophy induced by LIF and endothelin-1 (ET-1). A test compound that provides a positive response in the present assay would be useful for the therapeutic treatment of cardiac insufficiency diseases or disorders characterized or associated with an undesired hypertrophy of the cardiac muscle.

Cardiac myocytes from 1-day old Harlan Sprague Dawley rats (180  $\mu$ l at 7.5 x 10<sup>4</sup>/ml, serum <0.1, freshly isolated) are introduced on day 1 to 96-well plates previously coated with DMEM/F12 + 4%FCS. Test

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**FACSIMILE**

**Date:** October 7, 1998  
**To:** Ginger R. Dreger  
Genentech, Inc.  
**Fax Number:** 650-952-9881  
**From:** ATCC Patent Depository

**Number of pages:** 2  
(Including this page)

**REFERENCE:** Patent Deposit

pINCY-based plasmid DNA66672-1586 (Ref. PR1586) assigned ATCC 203265,  
pRK5E-based plasmid DNA71184-1634 (Ref. PR1634) assigned ATCC 203266,  
pINCY-based plasmid DNA66667-1596 (Ref. PR1596) assigned ATCC 203267,  
pINCY-based plasmid DNA66663-1598 (Ref. PR1598) assigned ATCC 203268,  
pINCY-based plasmid DNA66659-1593 (Ref. PR1593) assigned ATCC 203269,  
pINCY-based plasmid DNA73739-1645 (Ref. PR1645) assigned ATCC 203270,  
pINCY-based plasmid DNA58852-1637 (Ref. PR1637) assigned ATCC 203271,  
pINCY-based plasmid DNA66669-1597 (Ref. PR1597) assigned ATCC 203272,  
pRK5D-based plasmid DNA73401-1633 (Ref. PR1633) assigned ATCC 203273,  
pSPORT1-based plasmid DNA68879-1631 (Ref. PR1631) assigned ATCC 203274,  
pINCY-based plasmid DNA71290-1630 (Ref. PR1630) assigned ATCC 203275,  
pINCY-based plasmid DNA68864-1629 (Ref. PR1629) assigned ATCC 203276,  
pINCY-based plasmid DNA68874-1622 (Ref. PR1622) assigned ATCC 203277,  
pBluescript SKS-based plasmid DNA64842-1632 (Ref. PR1632) assigned ATCC 203278,  
pSPORT1-based plasmid DNA66660-1585 (Ref. PR1585) assigned ATCC 203279,  
pINCY-based plasmid DNA68871-1638 (Ref. PR1638) assigned ATCC 203280,  
pINCY-based plasmid DNA66674-1599 (Ref. PR1599) assigned ATCC 203281,  
pINCY-based plasmid DNA66675-1587 (Ref. PR1587) assigned ATCC 203282,  
pINCY-based plasmid DNA68866-1644 (Ref. PR1644) assigned ATCC 203283,  
pSPORT1-based plasmid DNA71269-1621 (Ref. PR1621) assigned ATCC 203284, and  
pINCY-based plasmid DNA71277-1636 (Ref. PR1636) assigned ATCC 203285.

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page 2

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An invoice will be sent under separate cover referencing P.O. 515441:

One time fee - 30 years	\$ 12,600.00
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Total amount to ATCC 203265-203285	\$ 15,750.00
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Barbara M. Hailey, Administrator  
ATCC Patent Depository

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